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Attorney Docket No. 21465-501 CIP DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Jonathan M. Rothberg, *et al.*	CONF. NO.:	8232
ASSIGNEE:	454 Corporation		
SERIAL NUMBER:	10/788,529	EXAMINER:	Young J. Kim
FILING DATE:	February 26. 2004	ART UNIT:	1637
FOR:	**METHOD OF SEQUENCING A NUCLEIC ACID**		

MAIL STOP AMENDMENT
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MARCEL MARGULIES UNDER 37 C.F.R. §1.132

I, MARCEL MARGULIES, declare and state that:

1. I am Vice President of Engineering, at 454 Life Sciences, the exclusive licensee of this application. My previous employment includes Director of New Technology Research at Perkin-Elmer's Instrument Division in Norwalk, CT, and Associate Director of the Hubble Space Telescope project.

2. I earned my B.Sc. in Engineering from the Free University of Brussels, in Belgium and a Ph.D. in theoretical physics from Columbia University.

3. I have read the specification and claims of the above-referenced patent application and the August 14, 2006 Office Action. I understand that there is a sole remaining rejection; the Examiner has rejected the claims as obvious over Chee et al., U.S. 2003/01808867 ("Chee") in view of Krull et al, WO 98/58079, ("Krull"). I provide this declaration to explain why the claims are not obvious over the cited art.

4. It is my opinion that the claimed invention provides a novel method of preparing an array for massively parallel, scalable sequencing platform that dramatically reduces the time, cost, space and sample preparation required for genome sequencing. The instant method therefore fulfills a long-felt but unmet need for large scale sequencing, and rapid whole-genome analysis.

5. In this patent application, the instant method provides a substrate comprising a cavitated fiber optic wafer formed from a fused bundle of a plurality of individual optical fibers, each individual fiber having specified dimensions (or to an apparatus having such a substrate). Specifically, each of the claims require that each individual optical fiber has a diameter between 3 and 100 μM, the thickness of the wafer (i.e., length of the optic fiber) between the top surface and the bottom surface is between 0.5 mm and 5.0 mm and the depth of each well ranges from between one half the diameter of an individual optical fiber and three times the diameter of an individual optical fiber. These specific parameters of the claimed cavitated fiber optic wafers are not chosen arbitrarily.

6. I have provided below data demonstrating that the claimed parameters of the instant method which provides a substrate comprising cavitated wafers perform differently from long fiber optical bundles disclosed in Chee and/or Krull. First, the claimed wafer thickness is important for light transmission properties of the claimed wafer. Second, the claimed well diameter and well depth parameters are important to the diffusion properties of claimed substrate.

7. With respect to the light transmission properties of the claimed wafer, I provide below data showing that light transmission through the individual fibers varies significantly as a function of the length of the fiber. As summarized in Figure 1, the transmission for a fiber optic measuring 300 mm in length is less than 18%. In contrast, nearly 100% transmission is achieved for an instantly claimed fiber optic measuring in length between 0.5mm and 5.0mm (I note that the data plotted does not take into account the fact that the actual mean path in any one fiber is longer than the linear fiber length -- which would exacerbate the differences be-

tween the short wafer thicknesses claimed and the long fiber bundles in the prior art).

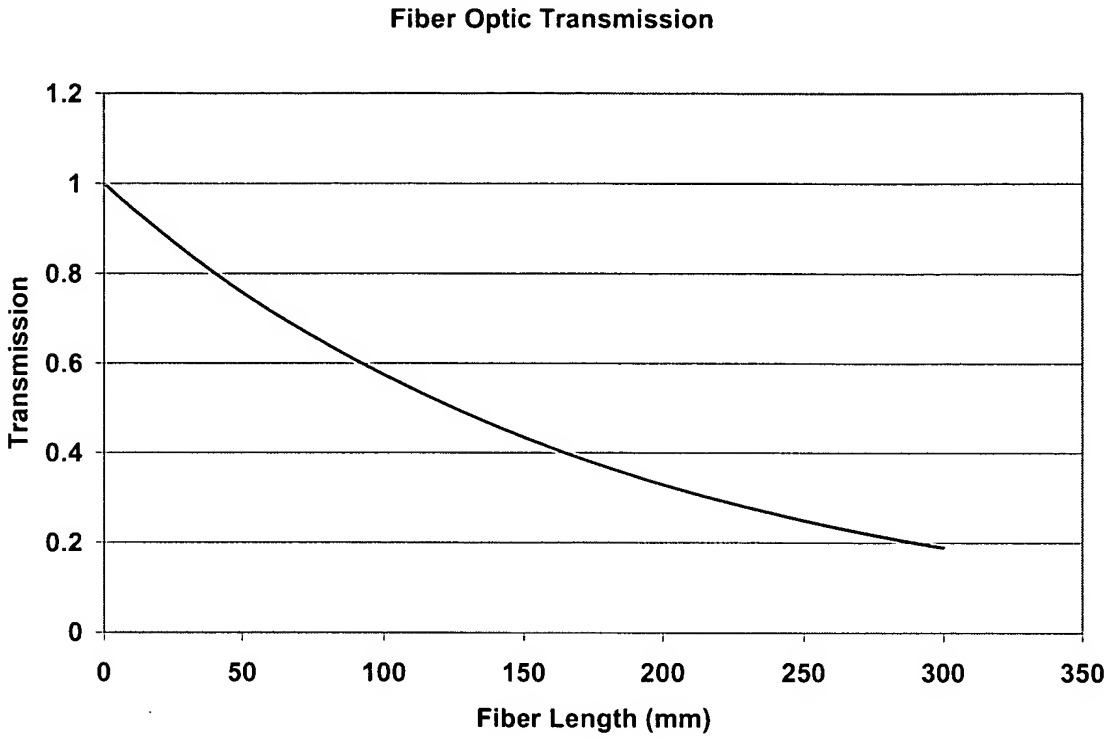

Figure 1.

8. Furthermore, the claimed parameters for well diameter (between 3 and 100μM) and well depth ranging from between one half diameter of an individual optical fiber and three times the diameter of an individual optical fiber are not arbitrarily chosen parameters. Well depth is selected on the basis of a number of competing requirements in a nucleic acid sequencing application: (1) wells need to be deep enough for DNA-carrying beads to remain in the wells in the presence of convective transport past the wells; (2) the wells must be sufficiently deep to provide adequate isolation against diffusion of by-products from a well in which incorporation is taking place to a well where no incorporation is occurring; (3) they must be shallow enough to allow rapid diffusion of nucleotides into the wells and rapid washing out of remaining nucleotides at the end of each flow cycle to enable high sequencing throughput and reduced reagent use; and (4) they must not be so deep that it would be easy for more than one bead to fit in a well.

9. To assess the sensitivity of this system to reaction by-products diffusing from one well into a neighboring one, a simplified one-dimensional model of interwell diffusion behavior was developed. We have found that at a well-to-well distance of 50 μm, diffusion of ATP produced during a pyrophosphate-based sequencing reaction, will induce a background signal on the order of 10% or less in an immediately neighboring well. The timescale for diffusion into and out of the wells is on the order of 10 s in this configuration.

10. We further created a one-dimensional model of the claimed fiberoptic cavitated wafer (i.e. modeled a linear array of wells) in which the wells are represented as lumped chemical reactors that produce pyrophosphate and ATP during the sequencing reaction. Within each well the generation of reaction by-products can be modeled by a set of coupled kinetic equations. Numerical solution of this set of equations is illustrated by Figure 2.

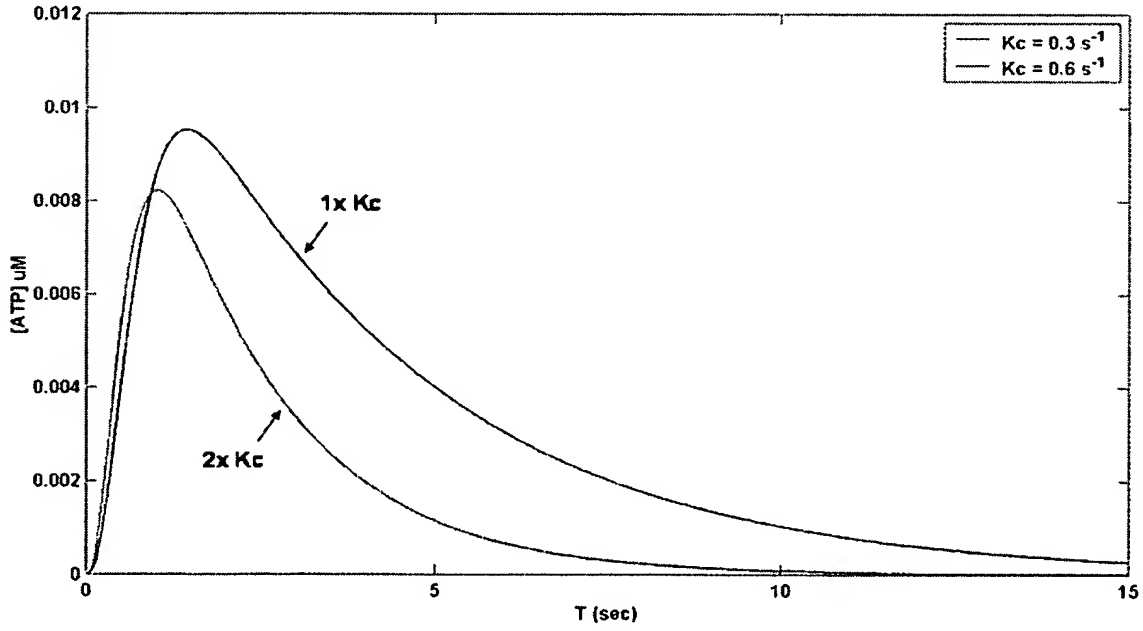

Figure 2.

11. As summarized in Figure 2, the simulation shows impact of well depth on signal generation and decay kinetics for a deeper (red) and shallower (green) well. The shallower well shows lower amount of signal (equal to the area under the curve) and faster signal decay (ATP leaves the well faster). Faster decay allows for reduction of flow cycle time interval, provided the signal generated is adequate. The deeper well results in a stronger signal (and slower signal decay) with the added caveat that too much signal, over too long a period of time, will result in deleterious optical bleed into neighboring wells. The effects of the above described observations on the experimental results of actual sequencing runs are summarized in Table 1.

Table 1.

Well Depth	40µm	35µm	30µm
Signal Per Base	1117 ± 267	1006 ± 226	787 ± 184
Diffusive signal bleed to down-stream well	13%	13%	17%
Doubly occupied wells	0.48%	0.27%	0.27%

12. In these experiments, three PTP's with different well depths (30 µm, 35 µm and 40 µm; all having a fiber optic diameter of approximately 35 µm) were used to sequence reference test fragments. As summarized in Table 1, the 40 µm deep wells show lower *chemical* cross-talk (signal bleed to downstream well). In contrast, the fraction of multiply occupied wells is twice as high as for 35 µm or 30 µm deep wells. The 30 µm deep wells have a low fraction of multiply occupied wells, but higher chemical cross-talk, and lower signal per base. Finally, the 35

μm wells have lower chemical cross-talk, a lower fraction of multiply occupied wells and adequate signal/base.

13. Table 2 summarizes actual sequencing results that bear out these findings.

Table 2

Well Depth	Key Pass	Read Error	Matching key pass reads over 100 bases		
			100%	98%	95%
40	17440	0.46%	81.84%	94.56%	96.78%
35	17928	0.40%	85.99%	95.29%	97.41%
30	17577	0.48%	79.19%	94.21%	97.21%

As summarized in Table 2, the read error is lowest for the 35 μm deep wells and the fraction of reads perfectly sequenced (matching at 100% over 100 bases) is highest for those wells. The results summarized in Table 2 unequivocally show the importance of the claimed well diameter and well depth parameters to achieve desired sequencing results. Neither Chee nor Krull teach or suggest the method comprising the use of wells with depth ranging from between one half the diameter of an individual optical fiber and three times the diameter of an individual optical fiber.

14. The Examiner cites Chee for obviousness but concedes that Chee does not disclose the method comprising the use of a cavitated fiber optic bundle as a wafer with thickness (i.e. length of the optic fiber) between 0.5mm and 5mm. The Examiner points to Krull to provide disclosure of a wafer (the Office Action cites to Krull, p. 13, lines 16-19). However, as discussed with the Examiner, Krull's optical "wafers" are completely unlike the optical fiber bundles claimed here (or the long fiber bundles referred to in Chee). Krull actively seeks to use the entire circumference of a single fiber for light transmission -- and to this end actually dissolves the external cladding from optical fiber pieces (see, e.g., Krull, Ex. 1, p. 43,

lines 7-10; Ex. 2, lines 20-23; Figure 4(b)). In contrast, in the instant cavitated fiber optic bundle wafer, light transmission through the fiber is desired; light transmission between fibers (prevented by the cladding) is to be minimized or avoided. For this reason, Krull teaches directly away from the instantly claimed wafer and thus teaches away from the instant method of using the substrate comprising the wafer for sequencing of nucleic acids, and for this reason cannot be combined with Chee.

15. As demonstrated above, Chee is silent as to the specifically recited dimensions of the cavitated fiberoptic substrate claimed here. And, the data presented here demonstrates that the claimed dimensional parameters were not arbitrarily chosen, but are important in determining the light transmission and diffusional characteristics of the claimed wafer.

16. In summary, neither Chee nor Krull, alone or in combination (and they cannot be combined) teach or suggest all the claimed elements, and there is no suggestion to modify Chee with Krull to obtain the claimed invention. For the reasons outlined here, I believe that Chee and Krull cannot make obvious the subject matter of the present claims.

17. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Dated: 1/15/07

Signed: _____
Marcel Margulies